coding sequence. This specific arrangement might allow the pros and cons of this mutation to be balanced to maximize the fitness of the plant, namely broad-spectrum pathogen resistance versus spontaneous necrosis. But how does this trick exactly work? The physical separation of the upstream and downstream promoters either by strong transcription termination signals or simply by increasing the distance in between the promoters seems to be generally sufficient to avoid interference [10,11]. The restoration of *Mlo* activity and concomitantly susceptibility by such separators could be efficiently analysed in the barley–powdery mildew interaction using transient assays as described before [12]. Such experiments would make the *mlo-11* allele an attractive model system not only for the control of pathogen resistance but also to understand the peculiar ways in which nature controls gene expression.

Acknowledgements

We are grateful to Alan Slusarenko and Sebastian Schornack for critical reading of the manuscript and excellent suggestions. We thank Ralph Panstruga and Paul Schulze-Lefert for their willingness to share unpublished observations. Markus Cavalar assisted us with the figures. Work in the laboratory of C.P. is supported by grants from the Deutsche Forschungsgemeinschaft (Pe819–1/2; Pe819–2/1; Kr589/13–2). Work in the laboratory of T.L. is supported by grants of the Deutsche Forschungsgemeinschaft (SFB 648 and LA 1338/2-2) and by the Kultusministerium des Landes Sachsen-Anhalt (3470A/902M).

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doi:10.1016/j.tplants.2005.04.005

Is there more than one way to attract a pollen tube?

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***ZmEA1* (*Zea mays egg apparatus 1*) is expressed only in the egg and synergid cells. Embryo sacs with presumed reduced expression of *ZmEA1* fail to attract pollen tubes. Together with data from *Arabidopsis* mutants and from elegant laser ablation experiments in *Torenia fournieri*, these results indicate that embryo sacs send signals to the incoming pollen tubes. We need to decipher how such signals are perceived and determine if the signals are species-specific.**

Embryo sacs beckon pollen tubes

Pollen tubes have a long journey from the stigma surface to the embryo sac. How do they find their way? During the early stages of pollen tube growth, sporophytic factors that are expressed in the stigma or style can provide guidance cues. Examples of such cues include transmitting tissue-

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Available online 30 April 2005

specific (TTS) protein [1] in tobacco, GABA (gamma-aminobutyric acid) in *Arabidopsis* [2] and chemocyanin [3] in lily. However, the haploid female gametophyte (Figure 1) can also send signals to attract the pollen tube; mutants that have delayed development of the embryo sac, such as *magatama* [4], fail to attract pollen tubes. Laser ablation experiments in *Torenia* [5] suggest that the source of attractant is the synergid cells but the molecular nature of the attractant in *Torenia* is not yet known. In the female gametophytic *Arabidopsis* mutants *feronia* [6] and *sirene* [7], pollen tubes are attracted to the mutant embryo sacs and enter the synergid but cannot burst to release the sperm cells; extra pollen tubes are attracted to the mutant embryo sacs, perhaps because production of the attractant from the synergids persists. Recently, Mihaela L. Marton *et al.* [8] presented evidence that a small peptide might be such an attractant in maize.

Thomas Dresselhaus's group [8] identified *ZmEA1* as an apparently abundant transcript in a cDNA library prepared from maize egg cells. The transcript is detected in egg cells and in synergid cells; it is progressively down-regulated after fertilization and is no longer detected in ten-day-old embryos. ZmEA1 is predicted to have one transmembrane domain. To determine subcellular localization for ZmEA1, the Dresselhaus group used a GFP-fusion protein. Because the GFP, fused at the C-terminus of ZmEA1, spread from the site of *ZmEA1* expression (egg and synergids) into the filiform apparatus (cell wall projections of the synergids) and into the surrounding nucellar cells, they suggest that the conserved C-terminus of the protein is cleaved from the transmembrane anchor to act as a diffusible signaling molecule. However, transient expression of the ZmEA1–GFP protein in onion cells, after particle bombardment, did not show GFP diffusion from the bombarded cell. If ZmEA1 is cleaved, perhaps the protease responsible is also embryo sac-specific. Visualization of GFP alone cannot reveal the nature of the putative cleaved product.

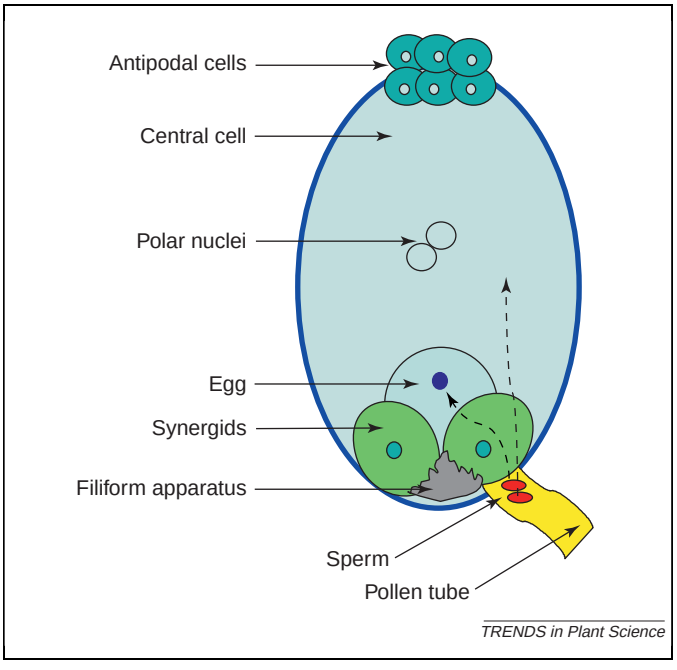

Figure 1. The embryo sac is the target for the pollen tube. The pollen tube grows through the stigma and style (not shown) to the ovary where it targets the embryo sac. The embryo sac comprises antipodal cells at the chalazal end of the ovule, a central cell in the center, and an egg cell and two synergid cells at the micropylar end of the ovule. The cell walls of the synergid cells are extensively invaginated and are termed the filiform apparatus. A pollen tube enters one of the two synergid cells and releases the sperm cells within the tube. One sperm cell fuses with the egg cell; the other sperm cell fuses with the central cell.

Transgenic experiments to test the role of ZmEA1

To address the possible role for ZmEA1, the Dresselhaus group used a transgenic approach. They generated RNAi lines and antisense lines in an inbred (A188) background (RNAi lines) or in a hybrid (A188×H99) background (RNAi lines and antisense lines). Most of these plants had multi-copy insertions at multiple loci. Two independent RNAi lines in the inbred background showed severe seed set problems; the five independent RNAi lines in the hybrid background showed only moderate seed set problems. Seed set in the antisense lines in the hybrid background ranged from severe to mild. For the RNAi lines there were four lines (two in each background) that served as controls; these had incomplete transgene integrations and showed no decrease in seed set. It was not reported if ZmEA1 transcript levels or protein levels were reduced in the antisense or RNAi lines, if there was a correlation with insert copy number, or if transcript levels or protein levels correlated with seed set impairment.

The Dresselhaus group selected two RNAi lines from the hybrid background (lines Rh6.1 and Rh15) with which to perform *in vitro* pollinations (*in vitro* pollinations use ovule sections that are excised from the ear and are then held in a moist environment). For the pollen donor for the *in vitro* pollinations, they used a transgenic line whose pollen was marked with a GUS reporter gene. Pollen was applied to the silks and then the fertilization status was scored 18 h later. When excised ovule pieces from the control (inbred A188) were pollinated, ~80% of the embryo sacs were fertilized, as judged after GUS staining (fertilized embryo sacs stain blue because the cytoplasm of the pollen tube is discharged into the embryo sac). However, when excised ovules from the Rh6.1 and Rh15 lines were stained for GUS, only ~50% of the embryo sacs stained blue, suggesting that fertilization had not occurred in the others. Closer examination of the non-blue ovules showed that the pollen tubes got close to the micropylar region but did not enter the synergid and discharge their contents. These images are compelling but do not explain why self-pollinations on the plant of these same two lines yielded nearly full seed set.

Monocot-specific signals?

Marton *et al.* [8] report that there are two genes in rice that are ~45% identical to ZmEA1; they show (in a supplemental figure in Ref. [8]) that a ZmEA1 probe detects similar sequences in rice, wheat and barley but not in two tested dicots (*Arabidopsis* and tobacco). Indeed, searches of plant EST databases and genomic sequences reveal that proteins similar to ZmEA1 are only present in

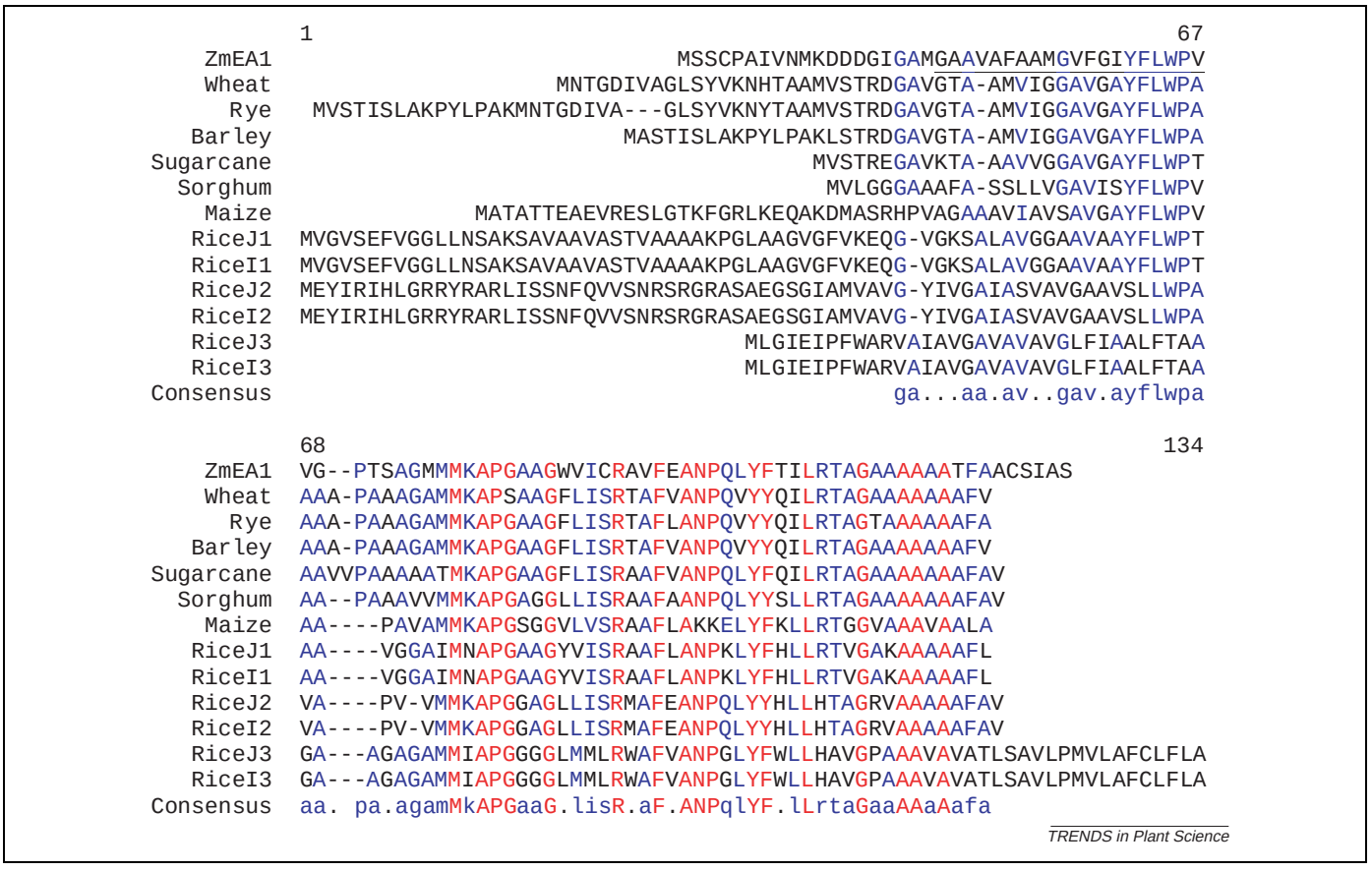

Figure 2. Alignment of ZmEA1 with predicted protein sequences from other monocots. Alignment with Multalin [11]. Red denotes highly conserved residues and blue denotes somewhat conserved residues. The predicted transmembrane domain in ZmEA1 is underlined. The GenBank accession numbers are: ZmEA1, accession no. AAW58117.1; wheat (EST from leaf), accession no. CK214516; rye, accession no. BE705426; barley (ESTs from shoot, root and leaf), accession no. CA027779; sugarcane (EST from leaf), accession no. CA298808; sorghum (EST from seedling), accession no. CD426610; maize (ESTs from embryo, root, seedling and silk), accession no. CD994779. Genomic sequences from rice subspecies (I, *Oryza sativa* ssp. *indica*; J, *Oryza sativa* ssp. *japonica*): J1 (XP_506453.1), J2 (XP_479095.1), J3 (AACV01006903.1), I1 (AAAA02022385.1), I2 (AAAA02022385.1) and I3 (AAAA02009265.1).

monocots. However, some of the ESTs are from diverse tissues, including leaves, roots or stems (Figure 2). If ZmEA1 is indeed a signaling molecule, the existence of similar proteins in other monocot tissues might imply a broader role for ZmEA1-like proteins. Marton *et al.* [8] also report that ZmEA1 sequences from different maize inbreds are only ~90% identical; this seems surprising given that the rice subspecies (*indica* and *japonica*) have sequences that are identical (Figure 2). It is possible that the divergent sequences observed in different maize inbreds are not alleles but instead represent one member each of duplicate genes [9].

There is still much to understand about ZmEA1. If ZmEA1 is indeed a pollen tube attractant, it would be informative to determine whether embryo sacs expressing the ZmEA1–GFP fusion protein attract more pollen tubes. Indeed, it is still not known whether the several distinct *Arabidopsis* mutants that yield similar phenotypes (e.g. lack of pollen tube attraction; multiple tubes attracted to one embryo sac) will resolve to single molecules. If there were multiple, redundant attractants one would predict that such mutants could not be found. Perhaps these mutants (and, by analogy, ZmEA1) identify a component of a multi-component signaling complex; the absence of any member therefore prevents signaling.

There are now significant numbers of ESTs from embryo sacs and/or their component cells from maize and wheat. The Dresselhaus group [10] has deposited ~400 ESTs from wheat eggs in GenBank and we have deposited >5600 ESTs from maize embryo sacs; summary annotations for the maize ESTs can be viewed at http://www.pgec.usda.gov/McCormick/McCormick/ResearchTopics/Gametes/Gametesindex.htm; they can also be retrieved from GenBank with the query '*Zea mays* embryo sac'. These databases are starting points from which to identify and then test other candidate signaling molecules (i.e. small secreted peptides or membrane-anchored peptides) for roles in pollen tube guidance. If each plant species emits only one signal from the embryo sac to attract the pollen tube, it would be interesting to determine whether transgenic replacement with one from another species could expand hybridization potentials.

Acknowledgements

Funding from USDA/ARS and from NSF Plant Genome grant 0211742 supported this work.

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1360-1385/$ - see front matter Published by Elsevier Ltd.
doi:10.1016/j.tplants.2005.04.001

Lipid microdomains – plant membranes get organized

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The plant plasma membrane is now known to be a more sophisticated structure than was previously thought. Sebastien Mongrand *et al.* and Georg Borner *et al.* have isolated specific plasma membrane microdomains ('lipid rafts') that are enriched in sterols and sphingolipids. These rafts contain distinct sets of proteins and might help to explain how plasma membrane proteins are positioned in certain parts of the cell to function in development and signalling.

Microdomains in plant plasma membranes

Being in the right place at the right time often determines positive outcomes in life. It holds true for plants. Plants operate strict spatio-temporal control of positioning of plasma membrane proteins to regulate development and physiology. For example, in the roots, the COBRA protein is held in lateral membranes to regulate lateral expansion [1], whereas auxin transporters PIN2 and AUX1, which are held in the apical membrane and the apical and basal membranes, respectively, direct vectorial auxin flow [2,3]. How is protein distribution achieved? Little attention has been paid to plasma membrane lipid composition as a mechanism for site-specific selection of a cohort of proteins. That is now set to change with the discovery of possible specialized microdomains ('lipid rafts') that can recruit specific proteins [4–8].

Lipids are exciting

Lipids play important roles in plants, most obviously as structural membrane components and energy stores. However, recently it has emerged that lipids also have roles as important signalling molecules in plant development and physiology (e.g. guard cell sphingolipid signal transduction). Molecular genetics has shown that the important lipids for development are those involved in brassinosteroid (BR) synthesis and those with independent roles [9]. BRs function as plant hormones, binding membrane-bound receptors and activating signalling cascades that regulate growth and development [10]. The inability of exogenous BRs to complement morphological mutants led to the identification of other important lipids. For example, the *hydra1* and *hydra2* mutants of *Arabidopsis* are defective in the synthesis of a sterol isomerase and a sterol reductase, respectively, resulting in altered lipid profiles, perturbed embryogenic patterns and underdeveloped roots, which cannot be rescued by BR application [11]. Such studies have led to suggestions that lipids influence plant function through other mechanisms. The most intriguing suggestion has been that non-random lateral separation of particular lipids leads to specialized microdomains in plasma membrane (rafts) that recruit proteins to specific areas and define domains of membrane function [12]. Lipid rafts have been isolated as non-ionic detergent-resistant membrane (DRM) fractions from animal and yeast plasma membranes [13–21]. Several research groups [4–7] have now isolated DRM fractions from plant plasma membrane and demonstrated a lipid and protein composition distinct from the plasma membrane as a whole.

Table 1 provides an overview of five papers that have contributed to our knowledge of plant lipid rafts. Although this article focuses primarily on the recent papers of Sebastien Mongrand [4] and Georg Borner [5] and their colleagues, their work was informed by earlier papers that provided the initial evidence of detergent-resistant

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Available online 5 May 2005